Neuron
Previews

Semaphorin Breaks Symmetry

Audrey S. Howell[1] and Kang Shen[1,*]
[1]Howard Hughes Medical Institute, Department of Biology, Stanford University, Stanford, CA 94305, USA
*Correspondence: kangshen@stanford.edu
DOI 10.1016/j.neuron.2011.07.020

Axon-dendrite polarity is likely instructed by extrinsic cues in the developing nervous system, though the mechanisms governing this process remain to be fully elucidated. In this issue of *Neuron*, Shelly et al. show that the axon guidance cue Semaphorin 3A can promote dendrite growth by inhibiting axon specification.

For information to flow through the nervous system, neurons must become subdivided into distinct axonal and dendritic domains. Given the importance of this process, neuronal polarity establishment has been a topic of intense study for many years. However, although many possible signaling pathways have been identified, relatively little is known about how a developing neuron interprets these cues to establish polarity.

Neuronal polarity could arise in vivo from two general mechanisms. A newly born neuron may contain internal positional information, perhaps inherited from the asymmetric division of its precursor cell. Alternatively, the environment surrounding the neuron may dictate the positions of the axon and dendrites through gradients of extrinsic signaling factors (reviewed in Barnes and Polleux, 2009). These mechanisms are not mutually exclusive, and external gradients may bias the activity of intrinsic signaling pathways. The ability of cultured rat hippocampal neurons to establish polarity in vitro in the absence of external cues has allowed for the experimental dissection of intrinsic neuronal signaling cues involved in establishing cell polarity. Dissociated rat hippocampal neurons display a stereotyped specification process, in which several neurites with no distinct identity initially emerge from the cell body, and, subsequently, a single neurite begins to rapidly grow and form the axon (Dotti et al., 1988).

The fact that axon emergence is one of the first observable polarization events, and that there is a single axon but multiple dendrites, has led to an axon-centric view of neuronal polarity establishment. In this view, a single neurite is specified as the axon, and all other neurites become dendrites by default. Therefore, most studies have focused on the signals specifying the axon, and relatively little is known about dendrite specification. One of the most important intracellular pathways shown to play a role in axon specification both in vitro and in vivo functions through the phosphorylation of LKB1 (Barnes et al., 2007; Shelly et al., 2007). LKB1 is the mammalian homolog of the *C. elegans par-4* gene, a gene with conserved roles in polarity establishment in many systems (McCaffrey and Macara, 2009). LKB1 is a serine/threonine kinase that is activated by association with the pseudokinase STRADα and PKA-dependent phosphorylation at S431 (Shelly and Poo, 2011). Following activation, LKB1 goes on to phosphorylate targets that help to polarize the cytoskeleton. Activated LBK1 accumulates in the growing axon, and loss of LKB1 results in a lack of axon formation, both in vitro and in vivo (Barnes et al., 2007; Shelly et al., 2007).

LKB1 may become locally activated through a rise in cAMP concentration in the neurite that will become the axon (Shelly et al., 2007; Shelly et al., 2010). Artificially raising the intracellular cAMP concentration with forskolin results in phosphorylation of LKB1 (Sapkota et al., 2001), as well as GSK-3β (Shelly et al., 2007), which has also been shown to play a role in axon specification (Barnes and Polleux, 2009). Local application of cAMP in vitro results in axon formation near the source of cAMP, as well as a decrease in cAMP and an increase in cGMP in other regions of the cell (Shelly et al., 2007; Shelly et al., 2010). The enzyme responsible for generating cGMP also appears to localize to the base of the developing apical dendrite of pyramidal neurons (Polleux et al., 2000). Therefore, the local concentrations of cAMP and cGMP may help to dictate axon and dendrite fate, respectively. The ability of a local increase in cAMP to suppress cAMP concentrations in other parts of the cell also presents an attractive mechanism for ensuring that only a single axon forms. What determines the local cAMP and cGMP concentrations? The most straightforward explanation is that external signaling molecules determine the internal gradient of cAMP and cGMP, but the in vivo evidence for particular extrinsic signals has been lacking.

An elegant study in this issue (Shelly et al., 2011) suggests that the well-established guidance cue Semaphorin3A (Sema3A) patterns the initial polarity of the neuron during early development. Specifically, Sema3A appears to locally inhibit axon differentiation. The authors plated dissociated hippocampal neurons on substrates coated with alternating stripes of various secreted factors implicated in neuronal polarization. By following the development of cells that adhered on the boundary of the stripes, they were able to compare the frequency of axon versus dendrite development when only a portion of the cell was exposed to the extracellular signaling factor. Axons appeared to preferentially form away from the Sema3A stripes, and dendrites preferentially differentiated on the Sema3A stripes, while in contrast, BDNF appeared to promote axon differentiation (Shelly et al., 2011).

Sema3A and BDNF appear to regulate neuronal polarity through cGMP and cAMP, respectively. Sema3A has previously been shown to use cGMP to direct the orientation of dendrite outgrowth in overlay culture (Polleux et al., 2000). In the current study, the authors use FRET reporters to show that bath application of Sema3A results in an increase in cGMP concentration, as well as a decrease in

cAMP, while BNDF has the opposite effect. Sema3A treatment also impaired forskolin-induced LKB1 and GSK-3β phosphorylation, consistent with the previous model of reciprocal regulation of cAMP and cGMP levels and the effect they have on axon development. Therefore, Sema3A may inhibit axon formation by impeding the cAMP-dependent phosphorylation of LKB1 and GSK-3β.

Does Sema3A regulate neuronal polarity in vivo? Shelly et al. used in utero electroporation of a RNAi construct to knockdown the expression of the Sema3A receptor NP1 in cortical neural progenitor cells and found that many of the resultant pyramidal neurons failed to migrate appropriately and instead remained multipolar (i.e., exhibiting multiple neurites) (Shelly et al., 2011). However, some neurons were able to migrate to the cortical plate and appeared to have established an axis of polarity. Interestingly, no gross effects on dendrite or axon differentiation were reported in the Sema3A knockout animals (Behar et al., 1996; Polleux et al., 1998), arguing that other extrinsic polarity cues can compensate for the loss of Sema3A.

A recent paper from Nishiyama et al. (2011) reached a similar conclusion concerning the effect of Sema3A on axon development in the *Xenopus* model system. In vitro Sema3A treatment resulted in the conversion of neurites that would normally form axons into dendrites (Nishiyama et al., 2011). The Nishiyama study adds an additional piece to the puzzle by suggesting that Sema3A-induced cGMP signaling is able to induce expression of functional $Ca_v2.3$ channels (Nishiyama et al., 2011). Expression of functional $Ca_v2.3$ channels was required for suppression of axonal development in vitro and for the appropriate acquisition of dendritic markers in vivo. Therefore, Sema3A may signal through a cGMP-mediated insertion of Cav2.3 channels to promote dendrite specification in addition to inhibiting axon specification.

Is the position of the axon purely dictated by a lack of inhibitory factors, or is there an extrinsic signal specifying axonal fate? Although BDNF could promote axon growth in vitro (Shelly et al., 2007; Shelly et al., 2010), in vivo evidence supporting its role in axon specification remains to be shown. Other signaling molecules have somewhat stronger support. Netrin is required for the appropriate outgrowth of the only neurite of the HSN neuron in *C. elegans* (Adler et al., 2006). In the absence of netrin (*unc-6*) or its receptor (*unc-40*), neurite outgrowth was delayed, and the process that did eventually emerge from the cell body was misguided (Adler et al., 2006). Signaling through the TGF-β receptor, TβR2, has recently been shown to be necessary for pyramidal axon formation in vivo (Yi et al., 2010).

A growing number of studies support the model that extrinsic signaling molecules can dictate the axon-dendrite polarity axis (Figure 1). While some molecules may promote axon outgrowth at the appropriate location, others such as Sema3A may promote dendrite formation by inhibiting acquisition of axonal fate. Other signals may be needed to help dictate appropriate dendrite outgrowth. The ability of neurons to break symmetry in vitro and the relatively low penetrance of in vivo phenotypes raise the possibility that these extrinsic cues may be redundant, with the internal polarizing pathways able to utilize a variety of extrinsic signals to dictate axon and dendrite outgrowth. As the signaling pathways regulating axon-dendrite polarity in vivo come into focus, it remains to be determined how these signals are spatially restricted or localized to effectively establish their cellular functions. Nonetheless, the study by Shelly et al. (2011) provides a novel framework within which to address these unresolved issues.

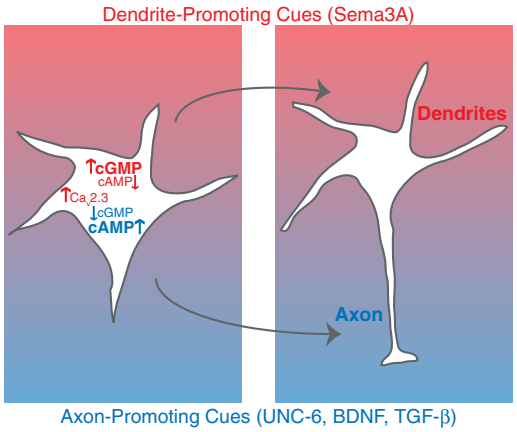

Figure 1. Extrinsic Factors Can Pattern the Developing Neuron
Dendrite-promoting cues, such as Sema3A, increase levels of cGMP and Cav2.3 channels, leading to local suppression of axon specification. Axon-promoting cues increase local cAMP while decreasing levels of cAMP elsewhere in the cell, leading to axon growth.

REFERENCES

Adler, C.E., Fetter, R.D., and Bargmann, C.I. (2006). Nat. Neurosci. *9*, 511–518.

Barnes, A.P., and Polleux, F. (2009). Annu. Rev. Neurosci. *32*, 347–381.

Barnes, A.P., Lilley, B.N., Pan, Y.A., Plummer, L.J., Powell, A.W., Raines, A.N., Sanes, J.R., and Polleux, F. (2007). Cell *129*, 549–563.

Behar, O., Golden, J.A., Mashimo, H., Schoen, F.J., and Fishman, M.C. (1996). Nature *383*, 525–528.

Dotti, C.G., Sullivan, C.A., and Banker, G.A. (1988). J. Neurosci. *8*, 1454–1468.

McCaffrey, L.M., and Macara, I.G. (2009). Cold Spring Harb Perspect Biol *1*, a001370.

Nishiyama, M., Togashi, K., von Schimmelmann, M.J., Lim, C.S., Maeda, S., Yamashita, N., Goshima, Y., Ishii, S., and Hong, K. (2011). Nat. Cell Biol. *13*, 676–685.

Polleux, F., Giger, R.J., Ginty, D.D., Kolodkin, A.L., and Ghosh, A. (1998). Science *282*, 1904–1906.

Polleux, F., Morrow, T., and Ghosh, A. (2000). Nature *404*, 567–573.

Sapkota, G.P., Kieloch, A., Lizcano, J.M., Lain, S., Arthur, J.S., Williams, M.R., Morrice, N., Deak, M., and Alessi, D.R. (2001). J. Biol. Chem. *276*, 19469–19482.

Shelly, M., and Poo, M.M. (2011). Dev. Neurobiol. *71*, 508–527.

Shelly, M., Cancedda, L., Heilshorn, S., Sumbre, G., and Poo, M.M. (2007). Cell *129*, 565–577.

Shelly, M., Lim, B.K., Cancedda, L., Heilshorn, S.C., Gao, H., and Poo, M.M. (2010). Science *327*, 547–552.

Shelly, M., Cancedda, L., Lim, B.K., Popescu, A., Cheng, P.L., Gao, H., and Poo, M.M. (2011). Neuron *71*, this issue, 433–446.

Yi, J.J., Barnes, A.P., Hand, R., Polleux, F., and Ehlers, M.D. (2010). Cell *142*, 144–157.